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A new species of *Marasmius* sect. *Sicci* from India

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Abstract — The marasmioid fungus *Marasmius midnapurensis* (*Marasmiaceae*, *Basidiomycota*) is described as a new species from India. Analysis of the internal transcribed spacer-1 region (ITS1) of the nuclear ribosomal RNA gene suggests that *M. midnapurensis* is phylogenetically distinct from closely related species and confirms its position within *Marasmius* sect. *Sicci*. Data on macro- and microscopic characters, habitat and comparisons with morphologically similar species are provided.

Key words — *Agaricomycetes*, biodiversity, phylogeny, taxonomy

Introduction

Marasmius Fr. (*Marasmiaceae*, *Agaricomycetes*, *Basidiomycota*), as traditionally accepted by Singer (1986), is polyphyletic (Wilson & Desjardin 2005). Based on the nLSU rDNA sequences, Wilson & Desjardin (2005) restricted the genus to a monophyletic lineage containing only sections *Marasmius, Sicci, Hygrometrici, Globulares, Neosessiles, Scotophysini,* and *Leveilleani* where taxa in *Sicci* and *Globulares* form a large joint clade (Wannathes et al. 2009). *Marasmius* sect. *Sicci* includes species characterized by a hymeniform pileipellis of broom cells of the *Siccus*-type and dextrinoid hyphae (Singer 1958, 1986).

Sixty-eight species and one variety have been reported thus far in India (Manjula 1983, Manimohan & Leelavathy 1989, Bilgrami et al. 1991). In the state of West Bengal only nine species of the genus have been reported: *Marasmius consocius* Berk., *M. erythropus* (Pers.) Fr., and *M. burkillii* (Massee) Manjula from the Darjeeling area (Berkeley 1851, Massee 1910); *M. pangerangensis* Henn., *M. campanella* Holterm., and *M. haematocephalus* (Mont.) Fr. from

Calcutta (Bose 1949, Bose & Chatterjee 1950, Roy 1953); *M. umbrinus* Pegler from Bankura district (Ray & Samajpati 1979); and *M. androsaceus* (L.) Fr. and *M. siccus* (Schwein.) Fr. from the lateritic region of West Bengal (Pradhan et al. 2011). All of these records should be revised in the light of modern taxonomic concepts and the many new species that have been described, especially from tropical regions.

The present paper describes *Marasmius midnapurensis*, a new fungal species from West Bengal.

Materials & methods

Basidiomata sampling and morphological studies

Basidiocarps of *Marasmius midnapurensis* were collected in 2011 during field trips to the state of West Bengal, India. Their morphology and ecology were noted and colour photographs were taken in the field. Microscopic features were obtained from dried material by mounting free-hand sections in 5% potassium hydroxide (KOH), Melzer's reagent, Congo red, or lactophenol-cotton blue and examination using a Carl Zeiss AX10 Imager A1 phase contrast microscope. Colour terms follow the British Fungus Flora Colour Chart (Anonymous 1969).The terms used to describe lamellae spacing are L for number of lamellae and l for number of lamellulae between two lamellae. Spore statistics include: X_m, the arithmetic mean of the spore length by spore width (± standard deviation) for n spores measured in a single specimen; Q, the quotient of spore length by spore width in any one spore, indicated as a range of variation in n spores measured; Q_m, the mean of Q-values in a single specimen; n, total number of spores measured; s, the number of specimens. The holotype collection has been deposited in the Calcutta University Herbarium (CUH).

DNA extraction, Polymerase Chain Reaction, and sequencing

Genomic DNA was extracted from dried (50°C) herbarium tissue (10–50 mg) using the 'Fungal gDNA Mini Kit' (Xcelris Genomics, Ahmedabad, India). The internal transcribed spacer-1 region (ITS1) was amplified using ITS1-F (Gardes & Bruns 1993) and ITS2 (White et al. 1990) primer pair. A hot start of 2 min at 94°C was followed by 30 cycles consisting of 30 s at 94°C, 1 min at 56°C, 1 min at 72°C, and a final elongation step of 5 min at 72°C. PCR products were checked on 2% agarose gel stained with ethidium bromide. PCR products were purified using QIAquick® Gel Extraction Kit (QIAGEN, Germany) and sequencing was done using Sanger methods. The obtained sequence generated was submitted to GenBank.

Phylogenetic analysis

Our new ITS1 sequence was submitted to GenBank (www.ncbi.nlm.nih.gov), and related *Marasmius* sequences were identified by a BLASTn search (http://blast.ncbi.nlm.nih.gov/). Sequences used in the phylogenetic analysis are indicated in Fig. 3, with *Mycena pura* (Pers.) P. Kumm. and *Marasmius rotula* (Scop.) Fr. as outgroup.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein 1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein

1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method (Jukes & Cantor 1969) and represent the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 142 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Taxonomy

Marasmius midnapurensis A.K. Dutta, P. Pradhan & K. Acharya, **sp. nov.** Figs 1, 2

MycoBank MB809410

Differs from *Marasmius jasminodorus* by its lighter colored and striate pileus, creamy lamellae forked towards the margin, non-strigose stipe base with whitish mycelium, slightly larger basidiospores, and the absence of a sweetish odor.

Type: India, West Bengal, Midnapur District, Ramnagar, Kasaphaltala, 21°43′29.9″N 87°31′36.6″E, 10 m asl., on dried *Acacia* leaves and on wood, 11 Aug. 2011, A.K. Dutta, P. Pradhan & K. Acharya (**Holotype,** CUH AMT002; GenBank, KF682470).

Etymology: specific epithet refers to the type locality.

Pileus 22–27 mm diam., broadly convex, sometimes umbonate, smooth, viscid when moist, light brown to light greyish brown with irregular light yellowish brown patches in the center, hygrophanous, smooth, striate. Context ≤0.8 mm thick, creamy, not changing colour when exposed. Lamellae L = 13–14, l = 3–4, adnexed, subdistant, forked towards the margin, creamy, regular, ≤4 mm broad, spacing ≤5 mm, margin even, concolorous. Collarium absent. Stipe 53–65 mm long, 2 mm broad overall, central, cylindrical, dark brick brown lower, creamy in upper part, equal, hollow, cartilaginous, strict to curved at lower portion, dry, smooth, flesh concolorous with the pileus, non-insititious, base covered with whitish mycelium. Odor and taste mild.

Basidiospores (10.7–)11.08–12.2(–15) × (3.5–)3.9–4.3(–4.7) µm [X_m = 11.57 ± 1.04 × 3.9 ± 0.39, Q = 2.5–3.5, Q_m = 3 ± 0.24, n = 150 spores (30 spores each among the collected 5 basidiocarps), s = 1 specimen], ellipsoid, slightly curved in profile, smooth, hyaline, inamyloid, thin-walled. Basidia (17.9–)23.2–23.6 (–24.4) × 4.7–6.8(–7.9) µm, clavate, hyaline, 4-spored, sterigmata 3.2–3.6 µm long. Basidioles 17.9–21.5 × 6.8–7.2 µm, fusoid to clavate, hyaline. Cheilocystidia common, of *Siccus*-type broom cells, main body 13.6–17.9 × 7.2–10 µm, cylindrical to clavate, hyaline, inamyloid, thin- to thick-walled, apical setulae 3.9–6.8(–10) × 1.4–1.8 µm, irregular in outline, obtuse to subacute, yellow to brownish yellow, thick-walled. Pleurocystidia absent. Pileipellis hymeniform, mottled, composed of *Siccus*-type broom cells, main

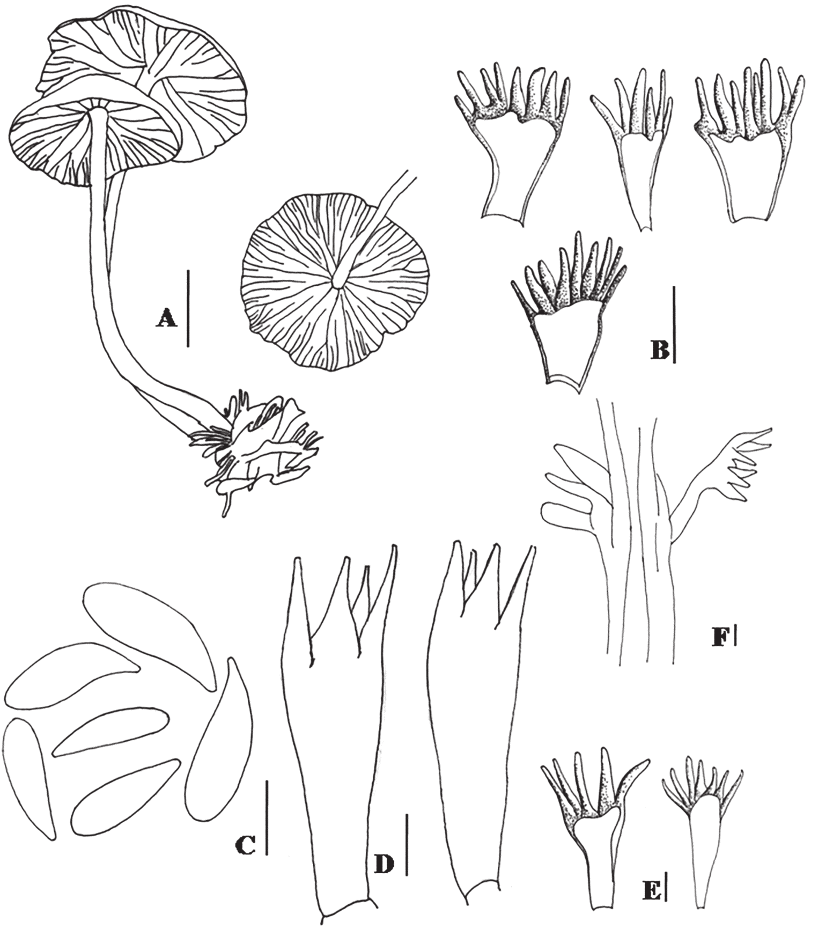

Figure 1. *Marasmius midnapurensis* (holotype): A. Basidiomes. B. *Siccus*-type cells of pileipellis. C. Basidiospores. D. Basidium. E. Cheilocystidia. F. Caulocystidia. Scale bars: A = 10 mm; B, F = 10 µm; C-E = 5 µm.

body 13.6–14.3 × (6.8–)7.1–10.3(–10.7) µm, clavate to broadly clavate, often branched, hyaline, inamyloid, thin- to thick-walled, apical setulae 3.9–6.8(10) × 1.4–1.8 µm, crowded, cylindrical to irregular in outline, obtuse to subacute, yellowish brown to brown, thick-walled, setae absent. Pileus trama interwoven, strongly dextrinoid. Lamellar trama hyphae interwoven, cylindrical to inflated, smooth, hyaline, dextrinoid, thin-walled, non-gelatinous. Stipitipellis hyphae 6–7 µm broad, parallel, yellowish brown to brown, smooth, dextrinoid, thick-walled, wall ≤0.7 µm thick, non-gelatinous.

Figure 2. *Marasmius midnapurensis* (CUH AMT002). Basidiomata. Scale bar = 10 mm.

Stipe trama hyphae parallel, hyaline, smooth, dextrinoid, thin-walled, non-gelatinous. Oleiferous hyphae present, ≤6.1 µm broad. Caulocystidia composed of two types of cells: a) *Siccus*-type broom cells with main body 28–29.4 × 3.5–4.3 µm, scattered, uncommon, irregular in outline, hyaline, apical setulae 6–6.4 × 1.8–2.1 µm, conical to wavy, pale yellow, thin- to thick-walled, b) non-setulose cells (11–)28.6–42.9(–50.1) × (3.5–)3.9–4.7(–6.3) µm, abundant, cylindrical or irregular in outline, seldom branched, obtuse to subacute, hyaline, inamyloid, thin- to thick-walled. Clamp connections present in all tissues.

Molecular & phylogenetic analysis

The amplified fragment of *M. midnapurensis* with the combination of primer set ITS1-F (forward) and ITS2 (reverse) produced 259 bp long stretches including the 226 bp ITS1 region. BLAST analyses with our *M. midnapurensis* sequences recovered *Marasmius* sequences, with the highest similarity shown by *M. jasminodorus* Wannathes et al. (92%), *M. araucariae* var. *siccipes* Desjardin et al. (89%), *M. aurantioferrugineus* Hongo (87%), *M.* cf. *cladophyllus* Berk. (87%), and *M. purpureostriatus* Hongo (84%).

Phylogenetic analysis of the ITS1 region inferred by the neighbor-joining method strongly supports *M. midnapurensis* as a distinct species within *Marasmius* and clusters *M. midnapurensis* and the seven other in-group

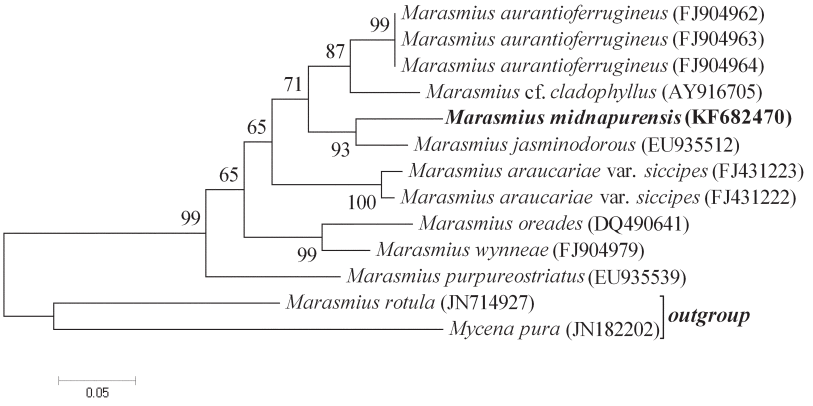

Figure 3. Phylogram showing relationship of *Marasmius midnapurensis* to closely related species of *Marasmius* based on internal transcribed spacer-1 (ITS1) sequences of rDNA. *Marasmius midnapurensis* is placed in bold font to highlight its phylogenetic position in the tree.

Marasmius spp. in a clade with 99% bootstrap support. Although *M. midnapurensis* forms a distinct branch sister to *M. jasminodorus* with 93% bootstrap support in the present study, another closely related species (*M. araucariae* var. *siccipes,* also in sect. *Sicci* ser. *Atrorubentes*) forms a separate clade with 65 % bootstrap support (Fig. 3).

Discussion

The absence of collarium and the presence of *Siccus*-type broom cells in the pileipellis, a well developed long central stipe, and adnexed lamellae suggest that *M. midnapurensis* belongs to *Marasmius* sect. *Sicci* Singer, while the presence of *Siccus*-type of broom cells combined with non-setulose cells on the stipe surface place it in the series *Atrorubentes* (Wannathes et al. 2009), where it appears to be closely related to *M. araucariae* var. *siccipes* described from Java, which differs in a more brownish orange pileus and darker lamellae (brownish orange to greyish brown), and slightly smaller basidiospores (8–12 × 3.5–4 µm; Desjardin et al. 2000).

Basidiospore shape and size, cheilocystidial and caulocystidial characters, and the absence of pleurocystidia are characters shared with *M. jasminodorus* from northern Thailand [ellipsoid basidiospores 9–14 × 3–4.5 µm with Q_m of 2.6–3.4, *Siccus*-type cheilocystidia (9–26 × 6–10 µm), caulocystidia of *Siccus*-type broom cells and cylindrical non-setulose cells, absence of pleurocystidia]. *Marasmius jasminodorus* differs macroscopically in its light brown to brownish pileus, pale yellowish white lamellae with pale brownish orange edges, a fragrant jasmine tea-like odor, and stipe base with strigose, brownish orange mycelium (Wannathes et al. 2009). Another similar species, *M. koreanus* Antonín et al.

Table 1. Comparison of *Marasmius midnapurensis* with similar *Marasmius* species.

Taxa	Pileus colour	Lamellae colour	Stipe base	Basidiospores (µm)	Cheilocystidia	Caulocystidia	Odor
M. midnapurensis	Light brown to light greyish brown; disc with irregular light yellowish brown patches	Creamy	Covered with whitish mycelium	10.7–15 × 3.5–4.7	*Siccus*-type	*Siccus*-type + non-setulose cells	Not distinctive
M. jasminodorus	Disc dark reddish brown; margin brown to brownish orange	Pale yellowish white	Strigose, mycelium brownish orange	9–14 × 3–4.5	*Siccus*-type	*Siccus*-type + non-setulose cells	Sweet, like jasmine tea
M. araucariae var. *siccipes*	Disc dark brown; margin brown to brownish orange	Brownish orange to greyish brown	Non-insititious	8–12 × 3.5–4	*Siccus*-type	*Siccus*-type + non-setulose cells	Not distinctive
M. purpureostriatus	Disc dark violet; sulcae greyish violet; elsewhere greyish yellow	Pale yellow	Non-insititious	19–30 × 4–7	Cylindrical to broadly clavate or pyriform	Absent	Not distinctive
M. aurantio-ferrugineus	Orange-ferrugineous	Pale yellowish white	Broadened	11.5–15 × 4–4.5(6)	Clavate, fusoid, subcylindrical, (sub) vesiculose	Cylindrical, (narrowly) clavate, (sub)fusoid	Not distinctive

from Republic of Korea, differs by brownish orange to reddish orange pileus with a distinctly brownish orange, rugulose centre and absence of caulocystidia (Antonín et al. 2012). All other species of *Marasmius* sect. *Sicci* described by Deng et al. (2012) from China differ from *M. midnapurensis* by the presence of setae on the pileus and only a single type of caulocystidia. In addition to the morphological differences (Table 1), the ITS sequence analysis clearly separates *M. midnapurensis* from *M. jasminodorus*, *M. aurantioferrugineus*, *M.* cf. *cladophyllus*, and *M. purpureostriatus*. Our findings support Tan et al. (2009) and Wannathes et al. (2009), who found that molecular data do not support morphologically delimited concepts within *Marasmius* groups. The objective of our study was to confirm the taxonomical position of *M. midnapurensis* (sect. *Sicci* ser. *Atrorubentes*) using ITS rDNA sequence data.

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